Single Molecule Energy Transfer Measurements: Application to Protein Folding

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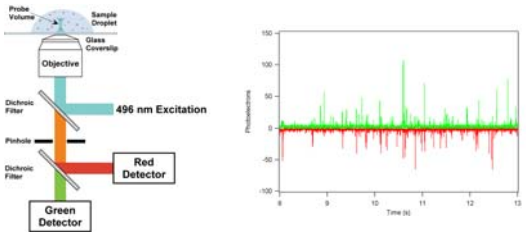
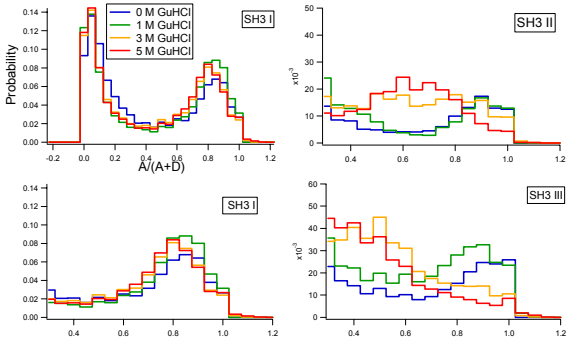
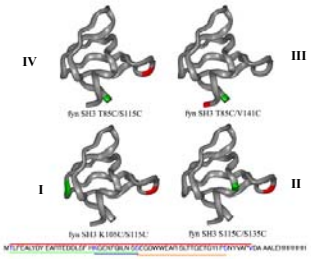
In recent years, a "new view" of protein folding has emerged, wherein a single folding path is replaced by multiple paths on an energy landscape that connect the unfolded and folded states. While this theoretical view has become popular, the experimental evidence for multiple folding pathways is sparse. Experimental observation of simple first order kinetics (two-state behavior) may belie the complexity of the folding/unfolding transition. Details of this transition may be elucidated by monitoring folding events and folding trajectories of individual protein molecules, an approach that we are pursuing using single-molecule spectroscopies. Here, we report on single-molecule fluorescence resonant energy transfer (FRET) measurements of a series of fluorescently labeled fyn SH3 domains under conditions that favor either the native or the unfolded state. We present energy transfer distributions determined from single molecule measurements and interpret the results ---- We also present methods capable of increasing the precision of single molecule energy transfer measurements.

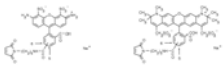
Single Molecule Fluorescence Resonance Energy Transfer Measurements in an open confocal volume element





fyn SH3 constructs





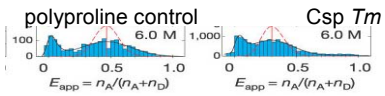
What can one learn from these measurements?

Average distance (better determined in bulk)

What about the width of the distribution?

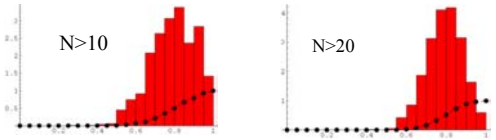
Narrower for the folded state than unfolded state

Some have taken it to be the distance probability distribution and extracted a potential of mean force
(Talaga et al. PNAS 2000; Deniz et al. PNAS 2000)



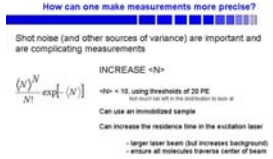
"**Probing the free-energy surface for protein folding with single-molecule fluorescence spectroscopy**," B. Schuler, E. A. Lipman, and W. A.Eaton *Nature*, Oct. 2002, p743-747

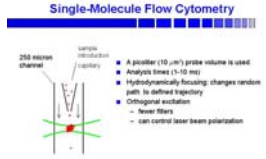
Are models correct for the distribution widths?

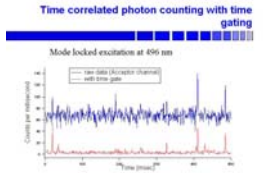


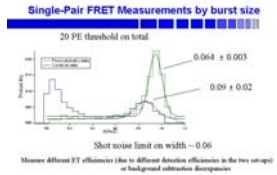
Simulated energy transfer distributions assuming donor and acceptor emission are Poisson processes

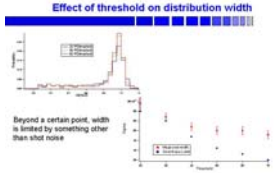
Single Molecule Flow Cytometry:
Increased precision and possibility of dynamics

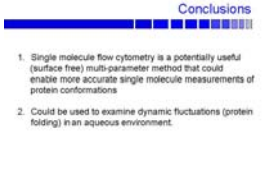












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